APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2005, p. 8752–8763 Vol. 71, No. 12
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.12.8752–8763.2005

Transcriptional Response of *Saccharomyces cerevisiae* to Desiccation and Rehydration†

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Received 24 May 2005/Accepted 10 August 2005

A transcriptional analysis of the response of *Saccharomyces cerevisiae* strain BY4743 to controlled air-drying (desiccation) and subsequent rehydration under minimal glucose conditions was performed. Expression of genes involved in fatty acid oxidation and the glyoxylate cycle was observed to increase during drying and remained in this state during the rehydration phase. When the BY4743 expression profile for the dried sample was compared to that of a commercially prepared dry active yeast, strikingly similar expression changes were observed. The fact that these two samples, dried by different means, possessed very similar transcriptional profiles supports the hypothesis that the response to desiccation is a coordinated event independent of the particular conditions involved in water removal. Similarities between "stationary-phase-essential genes" and those upregulated during desiccation were also noted, suggesting commonalities in different routes to reduced metabolic states. Trends in extracellular and intracellular glucose and trehalose levels suggested that the cells were in a "holding pattern" during the rehydration phase, a concept that was reinforced by cell cycle analyses. Application of a "redescription mining" algorithm suggested that sulfur metabolism is important for cell survival during desiccation and rehydration.

Water is essential for life, and thus the removal of even a small portion of the water from a cell is a severe, often lethal stress due to changes in cell volume and shape, condensation and crowding of the cytoplasm, membrane phase transitions, loss of supercoiling of DNA, oxidative damage, and metabolic arrest (5, 59). Organisms that can withstand the removal of virtually all of their cellular water possess a unique physiological adaptation that may have arisen very early in evolution, perhaps leading to a widespread distribution of primitive cells (50). The cellular responses to desiccation and subsequent rehydration are distinct from one another and are equally complex stresses (57). Survival of dehydration damage in some, but not all, organisms is correlated with the intracellular accumulation of compatible solutes, as well as vitrification of the cytoplasm. The mechanisms of action are described by the "water replacement hypothesis" and the glass transition theory, respectively, for which there is considerable experimental support (13–15).

Given the historical and economic significance of dried yeast and the current needs of the medical and biodefense communities for stabilized biologicals, it is surprising that there has been no genome-wide analyses of the response of yeast to water deficit. In an effort to fill this void, we analyzed the transcriptional response of yeast to desiccation and rehydration under glucose-limiting conditions. In addition, we compared the transcriptional profile of a dried lab strain (BY4743) with that of a dry active yeast purchased locally. Metabolite profiling was also performed to assess the roles that glucose and trehalose levels may have in the overall process. Due to the multiple stresses involved in desiccation and rehydration and our desire to compare the response of yeast to these stresses with those reported previously for heat shock, diauxic shift, etc., we applied a "redescription mining algorithm" to the gene expression data sets (52). Application of redescription mining shed new light on the mechanisms and processes involved in desiccation (stasis) and rehydration (recovery) in yeast.

MATERIALS AND METHODS

Yeast strains and growth conditions. The wild-type diploid strain *Saccharomyces cerevisiae* BY4743 (S288C) was obtained from the ATCC. The active dry yeast (composition and procedures used for drying are unknown) used for comparative purposes was purchased locally. Stock and seed cultures of strain BY4743 were grown in yeast extract-peptone-dextrose (YPD) medium (10 mg/ml yeast extract, 20 mg/ml Bacto peptone, 20 mg/ml glucose, pH 6.5 ± 0.2) at 30°C. Cultures were shaken at 150 rpm, with growth monitored by absorbance at 600 nm. Cells were grown from a frozen stock culture of 200 μl that was added to 50 ml of YPD medium in a 500-ml Erlenmeyer flask and incubated at 30°C overnight until cells reached an A_{600} of 1.5. A 6.25-ml aliquot of the cell suspension was used to inoculate 250 ml of YPD medium, and the culture was incubated at 30°C for approximately 7 h until the A_{600} reached 0.8. All manipulations prior to drying were performed with cells in the logarithmic phase of growth. Cells were centrifuged at 3,220 × *g* for 5 min. The supernatant fraction was discarded, the cell pellet was centrifuged once again, and any residual liquid was removed using a gel-loading tip. The cells were resuspended and dispersed in 140 ml of 1:20 diluted YPD medium at between 22 and 25°C. Different aliquots, which represent the control sample (time T_1), were removed for RNA extraction (5 ml), protein extraction (5 ml), carbohydrate analyses (2 ml), fluorescence-activated cell sorting (FACS) analysis (2 ml), and viable counts (100 μl). The remaining suspension was aliquoted into 9-cm-diameter glass petri dishes (15 ml/dish) for desiccation. The experimental procedures were completed in three independent trials under conditions that were, as far as possible, identical (Fig. 1).

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† Supplemental material for this article may be found at http://aem.asm.org/.

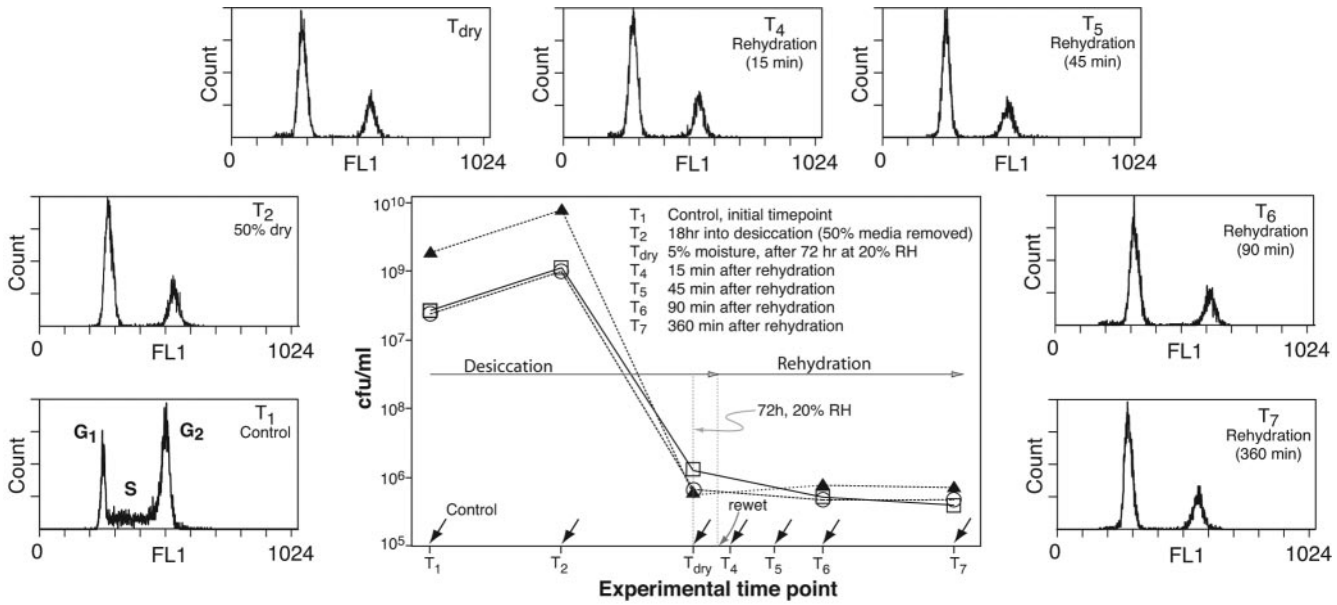

FIG. 1. Cell viability and cell cycle analysis of the desiccation and rehydration experiments. The number of CFU per milliliter is shown for each of the three experiments. The sample time points for transcriptional analysis are shown on the *x* axis and indicated by an arrow. The desiccation and rehydration phases are also indicated, as is the 72-h hold at 20% relative humidity (RH). The results of the FACS analyses (Sytox staining) are shown around the viability plot (clockwise from lower left).

Desiccation of cells. Desiccation was carried out in a custom-fabricated controlled atmosphere desiccation system at 22 to 25°C, where matric water potential (ψ_μ) was controlled via a psychrometer sensor and computer controller. The unit is comprised of a clean-room storage enclosure (Terra Universal, Irvine, CA) containing two individual closed-atmosphere compartments. Continuous replenishment of low-humidity atmosphere was accomplished with a nitrogen atmosphere controller (Terra Universal) using prepurified nitrogen. The system was retrofitted on site to add a closed-loop gas handling system, external gas conditioning chambers, environmental sensors, and a computerized controller with custom software. The closed-loop gas handling system is driven by two 0.07-m^3/min forced-draft fans and is designed with a gas equalization manifold to maintain equalized gas flow and moisture exchange across samples. The system permits the recirculation of nitrogen through modular external chambers containing desiccants or humidifying agents. A dual-cylinder regulator (Airgas) permitted the connection of two nitrogen cylinders for long-duration experiments. Computer control of humidity and temperature sensors (Pico Technology, St. Neots, United Kingdom) was used to refine environmental control parameters to a precision of ±0.01% and an accuracy of ±2% relative humidity. Software developed in house was used to monitor environmental parameters within the chamber. For more information on the unit and software, contact the corresponding author.

Nitrogen gas was circulated through a baffle system to achieve uniform drying within the chamber. Drying of cells was carried out at a ψ_μ of −163 MPa, where $\psi_\mu = 1{,}065T(\log_{10} p/p_0)$, T is temperature in degrees kelvin, and p/p_0 is water activity, which is numerically equivalent to relative humidity/100 (49). The relative humidity set point in this system was 30% ± 2%. A series of aliquots were withdrawn from the cell suspensions 18 h (time T_2) after the start of the drying process (Fig. 1). Samples were collected on a volume basis and were in the same ratio as collected at T_1. Once dry (42 h), the water potential was adjusted to −218 MPa (20% relative humidity) for a period of 72 h (this leads to complete desiccation of cells). A sample was collected at this time point, representing the dry sample (T_{dry}).

Hydration protocol/cell viability. Dry cells were hydrated (22 to 25°C) using 1:20 diluted YPD medium at 15 ml per plate. Cells were resuspended by gently rotating the plates in a circular motion. If needed, cells attached to the petri dish were released by gentle repeated pipetting. Resuspended cells from the four plates were pooled in a sterile 250-ml flask. Samples were collected for various analyses at time points of 15 min (T_4), 45 min (T_5), 90 min (T_6) and 360 min (T_7) after the start of rehydration. Aliquots of 100 μl were spread evenly on YPD agar (1.5%, wt/vol) plates incubated at 30°C, for a period of 48 h, before colonies were counted. Viability was assessed through serial dilution (10^{-1} to 10^{-5}) of cultures in sterile normal saline (0.85% sodium chloride, pH 7.2), at 22 to 25°C. The remaining cells were flash frozen in liquid nitrogen and stored at −70°C until further use.

Extraction of RNA. Total RNA was isolated essentially as described previously (55), with the following modifications. Cells (in 5-ml aliquots) were harvested by centrifugation, resuspended in 800 μl of AE buffer (50 mM sodium acetate, 10 mM EDTA, pH 5.3), and transferred to a 2.0-ml bead beater tube. Dried cells were directly suspended in AE buffer. Glass beads (total of 200 μl; each 0.5 mm in diameter), 80 μl of 10% (wt/vol) sodium dodecyl sulfate, and an equal volume of phenol (equilibrated in AE buffer) were added, and the cells were homogenized at 4°C for 1 min. Tubes were incubated at 65°C for 5 min and then chilled rapidly in dry ice-ethanol until phenol crystals appeared. At this point the mixture was centrifuged at room temperature (2 min). The aqueous phase was transferred to a new tube, an equal volume of phenol-chloroform (1:1, vol/vol; the latter contained isoamyl alcohol at 24:1, vol/vol) was added, and the contents were vortexed and spun for 5 min at room temperature in a tube containing phase lock gel (Phase Lock Gel Heavy; Eppendorf). This step was repeated two times. The aqueous phase was extracted once with an equal volume of 100% chloroform. The aqueous phase was then transferred to a new tube, 1/10 volume of 3 M sodium acetate (pH 5.3), and 2.5 volumes of ice-cold 100% ethanol were added, the contents were mixed, and nucleic acids were allowed to precipitate for 20 min at −20°C. RNA was recovered through centrifugation and washed with 80% (vol/vol) ethanol. The RNA pellet was air dried and resuspended in 100 μl of diethyl pyrocarbonate-treated water. RNA was quantified using a spectrophotometer, and its quality was assessed using the A_{260}/A_{280} ratio.

Gene expression analyses and data processing. Three experiments, with seven time points in each and three replicates per time point, required 63 Affymetrix Yeast S98 chips. each supporting probes for 6,400 open reading frames (ORFs), and 600 ORFs (some putative) from other strains (7,000 ORFs total). Hybridization and processing were performed at the University of Rochester Functional Genomics Center. Probe set signals were processed by a Wilcoxon's signed rank test and consolidated using a two-step linear mixed model. Relative expression estimates were derived with respect to both T_1 (six comparisons) and T_{dry} (four comparisons) (Fig. 1). This work was fully MIAME compliant. Specific details related to data processing are available in the supplemental material. A query-based website for viewing specific gene fold change data is also available (https://bioinformatics.cs.vt.edu/~vsinghal/yeast).

Redescription mining. Redescription utilizes an alternating algorithm (52) in which decision trees are used as representations of set-theoretic expressions involving "descriptors." In each iteration, the algorithm is given a decision tree as input and aims to grow (learn) another tree to match the set expressions

defined by the former; this new tree is then used as input for the next iteration. Thus, at any point, the trees are matched at possibly some (or all) of their leaves. The algorithm terminates when a certain desired number of redescriptions have been mined or when a given coverage of the underlying set has been obtained. Central to this process is the definition of a "universal set of genes" (here, chosen to be the 210 ORFs that are fourfold positively or negatively regulated in some time point) and a vocabulary of descriptors that qualify different subsets of this universal set. The strength of the redescription is given by Jaccard's coefficient, which is the ratio of the number of genes common to both sides of the redescription to the number of genes involved in either side. An enhanced description of the process is available in the supplemental material, including the vocabulary of descriptors (see Table S1 in the supplemental material) and a listing of the universal gene set (see Table S2 in the supplemental material).

Flow cytometry. FACS for cell cycle analysis followed established procedures (39) and was modified as follows. Cells were harvested by centrifugation at 3,220 × *g* for 5 min, and pellets were resuspended in 1.5 ml sterile water, followed by the addition of 3.5 ml of 95% (vol/vol) ethanol. The fixed cells were stored at 4°C until further use. For staining, a 500-μl aliquot of the suspension was centrifuged, and the recovered pellet was resuspended first in 1 ml of water and then, after centrifugation, in 0.5 ml of 50 mM Tris-HCl, pH 8.0. RNase A (10 μl of a 10-mg/ml solution) was added, and the cells were incubated for 4 h at 37°C and then kept overnight at 4°C. The cells were recovered by centrifugation, and resuspended in 0.5 ml 50 mM Tris-HCl (pH 7.5) containing 2 mg/ml proteinase K, and incubated at 50°C for 30 min. The cells were then recovered and resuspended in 0.5 ml of FACS buffer (200 mM Tris-HCl, 200 mM NaCl, 78 mM $MgCl_2$, pH 7.5). A 100-μl aliquot was transferred to a new tube, and 1 ml of 1× Sytox Green (S-7020; Molecular Probes, Eugene, OR) solution in 50 mM Tris-HCl (pH 7.5) was added, providing a final Sytox Green concentration of 1 μM. The cells were analyzed using a flow cytometer (Beckman Coulter EPICS-XL) with gating to observe the living cells. The software used for data analysis was EPICS version 1.5.

Glucose and trehalose determinations. Cell and supernatant fractions were separated from 1-ml aliquots by centrifugation at 20,800 × *g* for 10 min at 4°C, with the resulting fractions flash frozen in liquid nitrogen and freeze-dried. Intracellular glucose was determined by the boiling water method (44), and intracellular trehalose was determined by the boiling bicarbonate protocol (29). All final glucose assays utilized the glucose oxidase/peroxidase kit (Boehringer Mannheim), following the directions of the manufacturer. Extracellular glucose concentrations were measured directly with the glucose oxidase/peroxidase kit, while extracellular trehalose determinations required a bicarbonate treatment and subsequent trehalase hydrolysis prior to the glucose measurement.

Measurement of cell water content. Intracellular water content was determined by thermogravimetric analysis as outlined previously (2) with the following modifications. Desiccated cells were harvested by scraping them from the surfaces of the glass petri dishes with a surgical blade. The powder was transferred to a 10-mm-diameter platinum pan, and analysis was conducted with a TA-Instruments TGA 2950 thermogravimetric analyzer. Starting from room temperature, with a heating rate of 10°C/min, the temperature was raised to 60°C. When the mass loss rate was practically zero, the temperature was raised to 125°C for 7 min in order to complete the water elimination and to determine the water-free residual mass.

Nucleotide sequence accession numbers. Data from this work have been deposited at the GEO website (accession numbers GSE1311 to -1314).

RESULTS

Growth and viability. The mode of growth, recovery, and profile of viability of the BY4743 strain after imposition of stress were consistent in three independent trials (Fig. 1). The cell suspension at the onset of drying included subpopulations of cells in the G_1, S, and G_2 phases of the cell cycle, with cell moisture contents of 58%. After drying commenced, the cells continued to grow and divide for at least 18 h, at which time approximately 50% of the medium had evaporated (Fig. 1). There was minimal glucose detected in the medium at the T_2 time point, and cell cycle analysis revealed a dramatic shift to cells predominantly in G_1. The moisture contents of the dried samples (T_{dry}), after 72 h of storage at 20% relative humidity were 5% by weight. Only the G_1 and G_2 phases were detected after rehydration, and the G_1/G_2 ratio remained approximately the same throughout the remainder of the experiment. These cells presumably had adapted to desiccation, as there was little to no further loss of viability after 72 h of storage and addition of fresh medium. In separate experiments, it took approximately 44 h for cells to acquire a normal FACS profile similar to that of the T_1 sample (Fig. 1) in 1:20 YPD at 25°C; with full-strength YPD at 30°C, the recovery time was approximately 13 h (data not shown).

Comparison of BY4743 and commercial dry active yeast transcriptomes. The traditional method for verification of microarray data sets is by quantitative PCR. Generally, a subset of genes identified as undergoing a significant fold change are assessed for expression levels by quantitative PCR and the results compared to those obtained in the microarray experiments. We approached verification in a different manner: the gene expression levels (fold change) of a commercial dry active yeast (dried state) and desiccated *S. cerevisiae* BY4743 (T_{dry}) were compared using the T_1 expression level of BY4743 as the baseline, deriving a scatter plot for probes with significant fold changes (Fig. 2). Our reasoning for this approach was twofold. If the transcriptional profiles were similar, not only would the microarray data be verified but, more importantly, the data would support the hypothesis that the transcriptional response to desiccation is independent of yeast strain and drying regimen.

There was a very strong correlation between the responses of each transcriptome to desiccation. The bottom panel of Fig. 2 shows the most up-regulated genes in both samples; *FDH1* (encoding formate dehydrogenase) and *SIP18* (encoding a putative phospholipid binding protein) showed the highest fold changes in both data sets. Fewer than 5% of the probes had conflicting responses (i.e., positive in one and negative in another; a list of genes with fold change differences between the dry active and BY4743 yeasts of greater than three can be found in the supplemental material). These results verify the microarray data sets and show that two different strains dried by two different means yield very similar transcriptional responses to desiccation. This strongly supports the hypothesis that the desiccation and rehydration responses of yeast are stringent and coordinated.

Two genes that showed discordant behavior were *MAL32* and *YGR287C*. Transcripts for both of these genes were in higher abundance in the commercial yeast than in BY4743 (Fig. 2). These genes are annotated as encoding maltases, which release glucose from the disaccharide maltose. These genes may be constitutive in industrial yeasts, where the growth medium typically contains maltose. Two other genes with significant differences in expression levels in the two data sets are *SUC4* and *RTM1*, encoding a sucrose-hydrolyzing enzyme and *r*esistance *t*o *m*olasses, respectively (Fig. 2). Expression of *SUC4* may be in response to the composition of the medium; the *RTM1* gene is not present in BY4743.

Desiccation and rehydration processes are similar to stationary-phase growth. The expression levels for the vast majority of genes that were up- or down-regulated during desiccation and rehydration shifted to a new level during the evaporation phase of the experiment (T_1 versus T_2) and persisted at that level throughout the remainder of the experiment. These results as well as the viability and cell cycle data

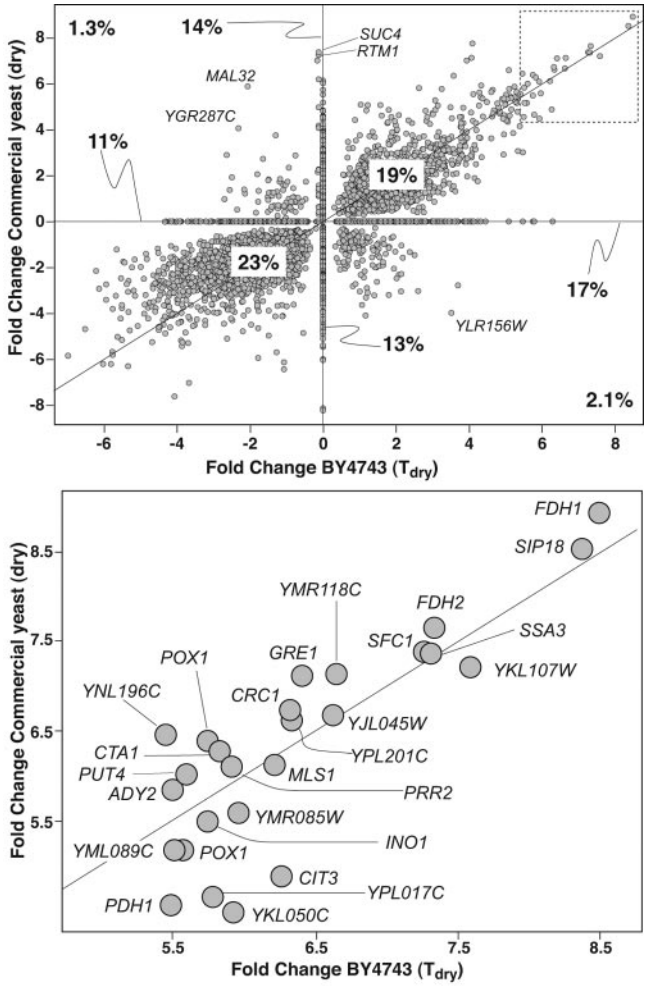

FIG. 2. Comparison of fold changes between commercial dry active yeast and the BY4743 strain at T_{dry}. The top panel shows each probe with a significant fold change (relative to T_1) in both data sets. The percentage of probes with particular responses is also listed (see text for details). The bottom panel is an expanded view of the most highly expressed probes in both data sets, with the specific genes associated with each probe identified.

(Fig. 1) support a hypothesis that the cells were in a "holding pattern" during rehydration, i.e., a loss of cells in S phase and concomitant increase in cells in quiescence (G_0/G_1). Entry into the quiescent state can also be induced by caloric restriction (35) or by reaching the stationary phase of growth (25, 40). A transcriptional analysis of stationary-phase growth identified 127 genes essential for survival at 37°C (40). More than half of these genes were found to be up-regulated more than twofold during desiccation and rehydration. These genes included *CIT3*, *CRC1*, *CTA1*, *FDH1*, *GRE1*, *MLS1*, *SFC1*, and *SIP18* (Fig. 2). While the stationary-phase essential genes were enriched with genes associated with oxidative phosphorylation (17 identified), our data set did not show similar expression level increases for this gene class. This would suggest that the desiccated and rehydrated cells were not energy depleted but had adapted to the environment. Only 6 of the 127 stationary-phase essential genes showed more than a twofold decrease in expression during desiccation and rehydration. Two of these were *GAC1*, a gene coding for a protein phosphatase that regulates glycogen synthesis, and *HSP30*, which encodes a stress-responsive protein that negatively regulates the H^+-ATPase Pma1p (18).

Transcriptional changes related to carbon and fatty acid metabolism. Genes related to fatty acid catabolism, gluconeogenesis, and the glyoxylate cycle were activated during desiccation and subsequent rehydration of strain BY4743 (Fig. 3). While at present it is not possible to define the metabolic shift as being related to only glucose limitation or desiccation, similar expression levels were also observed for the dry commercial yeast sample, suggesting that such metabolic shifts are a component of the desiccation process. Many of the genes associated with glucose limitation have been shown to be under Snf1p, Adr1p, and Cat8p control (27, 56, 67, 68), and indeed most of these genes were up-regulated and remained that way through the rehydration phase.

There are two pathways for the transport of acetyl units from peroxisomes to mitochondria, via the glyoxylate cycle or Cat2p-dependent carnitine transport (62). All of the genes encoding components of the glyoxylate cycle (*CIT2*, *IDP2*, *ICL1*, *MLS1*, *CAT2*, *YAT1*, and *CRC1*) were up-regulated as much as sevenfold, with the single exception of *MDH2*, encoding malate dehydrogenase (no change in transcription). Succinate, derived from the glyoxylate cycle, enters the mitochondrion via the mitochondrial carrier protein Sfc1p; *SFC1* showed greater than sevenfold up-regulation for all time points (Fig. 2, bottom panel).

It has been proposed that *CIT3*, *PDH1*, and *ICL2* make up the methylcitrate cycle in *S. cerevisiae*, providing succinate and pyruvate from propionyl coenzyme A (propionyl-CoA) and oxaloacetate (19, 38, 41). As both *PDH1* and *ICL2* transcripts were up-regulated in our studies (Fig. 3), it is highly probable that the methylcitrate cycle is in operation during desiccation and rehydration. Propionyl-CoA is formed from beta-oxidation of odd-numbered fatty acids, as well as degradation of amino acids such as isoleucine. A highly up-regulated methylcitrate pathway would reduce the requirement for the genes found to be unchanged or down-regulated in the tricarboxylic acid cycle (i.e., *FUM1*, *KGD1*, and *MDH1*).

Fold change analysis reveals a conspicuous increase in *IDP2* and *IDP3* (cytosolic isocitrate dehydrogenases) without concomitant increases in *KGD1* and *KGD2* (α-ketoglutarate dehydrogenases) (Fig. 3). The cellular concentration of α-ketoglutarate may be modulated by the changes in transcription of the three known glutamate dehydrogenase genes, *GDH1* (down 2-fold), *GDH2* (up 2.5-fold), and *GDH3* (up as much as 6.7-fold). The enzymes encoded by *GDH1* and *GDH3* are NADP dependent and catalyze the synthesis of glutamate from ammonia and α-ketoglutarate. *GDH2* is NAD dependent and catalyzes the reverse reaction (17). Interestingly, *YHR033W*, encoding a putative γ-glutamyl kinase, is also up-regulated, from 2.5-fold at the first time point to as much as 5.6-fold at the last time point. These findings support the hypothesis that α-ketoglutarate levels are important in regulating flow through the pathways for nitrogen recycling, energy production, and gluconeogenesis (41). Similar trends were also observed in chemostats under glucose-limiting conditions (8).

A link between pyruvate and formate dehydrogenase? The putative formate dehydrogenase genes *FDH1* and *FDH2* were

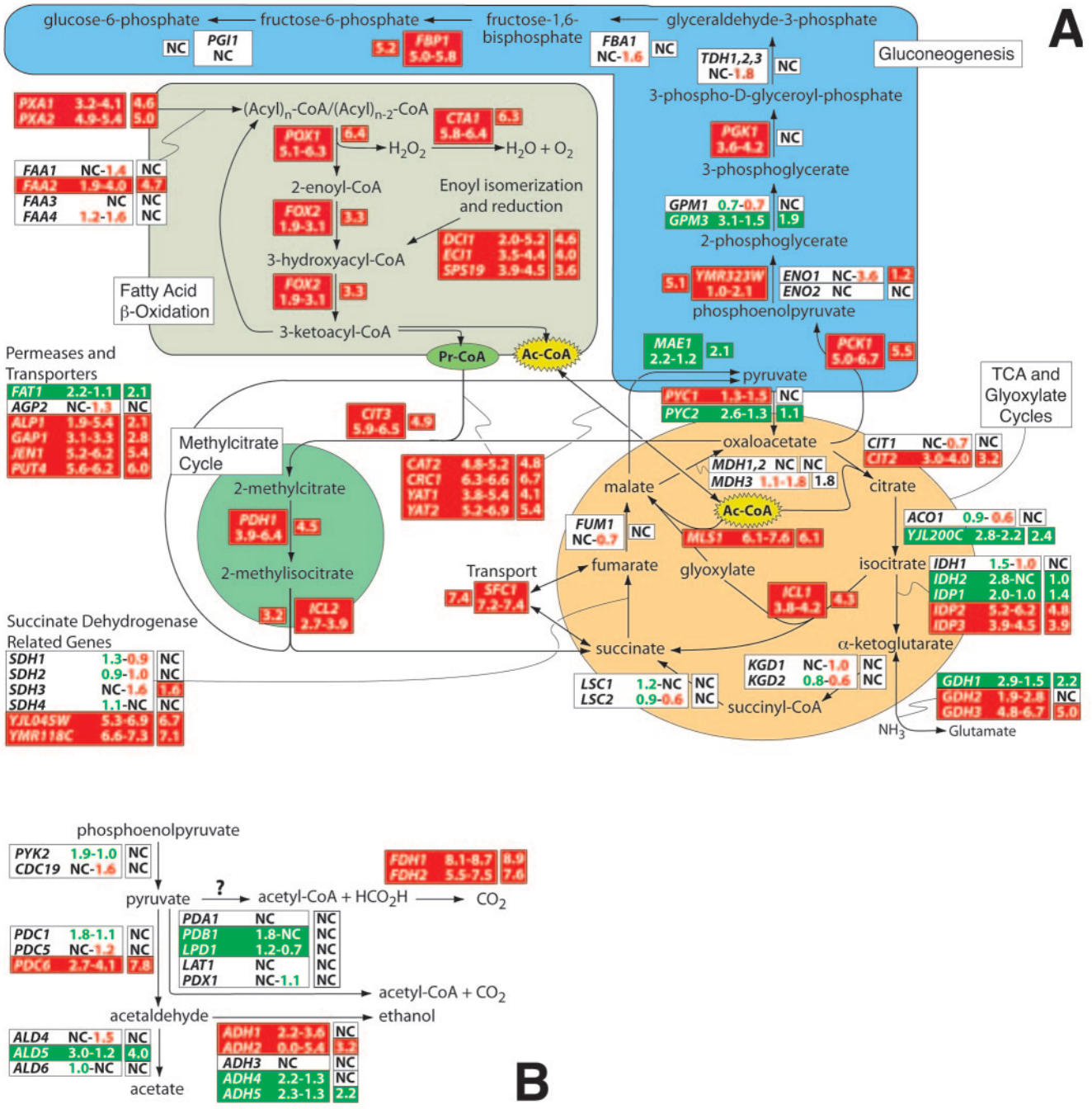

FIG. 3. Transcript changes in the metabolism genes of *Saccharomyces cerevisiae* as a result of the desiccation and rehydration regimen. Genes are shown in color-coded boxes: red, up-regulation; green, down-regulation; white, little to no change. The numbers in each box are the transcript level fold change ranges over the entire course of the experiment relative to the control time point (T_1); values within the green boxes are negative, whereas those in the white boxes are color-coded for negative (green) and positive (red) fold changes. NC, no change. TCA, tricarboxylic acid. The smaller boxes adjacent to each gene set show the fold changes obtained for a commercial dry active yeast. A, broad overview; B, focus on pyruvate-related processes. Access to all data is at https://bioinformatics.cs.vt.edu/~vsinghal/yeast/.

two of the most up-regulated genes present throughout the course of the experiment (Fig. 2). Assuming that these genes do in fact encode active formate dehydrogenases, where is the formate coming from? Formate is most likely generated from one of the three pathways that break down pyruvate to 2-carbon (acetate, acetyl-CoA) and one-carbon (formate and CO_2) products (Fig. 3B). Many prokaryotes and eukaryotes possess pyruvate-formate lyase (PFL), also known as formate *C*-acetyltransferase (EC 2.3.1.54). This enzyme is S-adenosylmethionine (SAM) dependent, proceeding by a free radical pathway that is strongly inhibited by oxygen (3). As the formate dehydrogenases have also been shown to be up-regulated under

carbon-limited, normoxic conditions in yeast (7), the possibility exists that the enzyme that performs the pyruvate-formate lyase reaction may proceed by an alternative, radical-free mechanism.

Cell wall-related genes. Desiccation and rehydration present yeast cells with a combined change in water potential and osmolarity. Stresses will develop in the cell wall during imposition of both stresses that must be alleviated, or the wall's integrity may be compromised. Changes in polysaccharide ratios, changes in glycoprotein levels, and/or a redistribution of synthesis/repair machinery can occur in the cells to minimize stress development (60). The microarray analyses revealed a dramatic drop in the transcripts encoding the β-(1-3)-glucan synthase, *FKS1*. Numerous cell wall mannoprotein-related genes were also down-regulated, including the protein *O*-mannosyltransferase genes *PMT1* to *-5*, which would suggest a loss of rigidity in the cell wall (37).

A transcriptional analysis of several cell wall null mutant strains (*FKS1*, *KRE6*, *MNN9*, *GAS1*, and *SMI1* mutants) was reported recently (32). A set of 80 up-regulated genes was identified as a cell wall compensatory cluster, being up-regulated in all the aforementioned mutant strains. In comparing the expression levels of this compensatory cluster with our data, we see very little correlation; 38 genes are up-regulated, 35 are down-regulated, and the remainder are unchanged. Of the 190 transcripts up-regulated in the *FKS1* null mutation study (32), only 25% of them were found to be up-regulated (greater than onefold) during desiccation and rehydration, with most of these genes being metabolism related. Interestingly, several of these *FKS1* mutant transcripts that were significantly down-regulated in our study were directly related to cell wall organization, including *EXG1* (exo-1,3-β-glucanase gene; 4-fold), *SCW10* (putative glucanase gene, 4.5-fold), and *CWP1* (cell wall mannoprotein gene, 3.5-fold).

Attenuation of environmental stress response. The majority of the genes classified in the environmental stress response showed no change in transcription in response to either desiccation or rehydration. These included *SOD1* and *CTT1*, encoding Cu-Zn superoxide dismutase and catalase, respectively. In other desiccation-tolerant organisms, as well as the dauer stage of *Caenorhabditis elegans*, the transcription of *SOD* genes and translation of *SOD* mRNA are highly active (58, 63). Several environmental stress response genes, including those for glycogen phosphorylase (*GPH1*) and a protein induced by heat shock, ethanol treatment, and entry in stationary phase (*HSP30*), were in fact down-regulated more than twofold. Of the four genes that did show enhanced transcription (*ECM4*, *YIL057C*, *OM45*, and *UBC8*), *YIL057C*, of unknown function, was up-regulated the greatest (4- to 5.6-fold).

Multistress environments and redescription analysis. Determining response networks for organisms exposed to multiple simultaneous stresses, such as those encountered during desiccation and rehydration experiments with minimal glucose, is much more complex than it is for single-stress experiments. Overlapping response networks make the assignment of mechanism difficult. One approach to model multistress responses is to relate the multistress data sets to the available single-stress data sets via a "redescription" process. The value of such an analysis is the ability to uncover the components of overlapping and parallel pathways, thus determining the commonality in the response of cells to specific environmental stressors.

The inputs to the redescription mining algorithm are (i) a set of genes and (ii) different vocabularies for defining subsets of the given set (52). For our work here, we defined the set of genes as the 210 yeast ORF "high expressors," i.e., those genes that exhibited fold changes (positive or negative) of greater than 5 for any experimental comparison or had an average expression of fourfold (positive or negative) or greater across all comparisons. The vocabularies are some biologically meaningful way to define subsets (called descriptors) of this universal set. Examples of descriptors would be statements such as "genes that are expressed twofold or more in the dried yeast" or "genes that belong to gene ontology category cell wall (sensu Fungi)." The first descriptor is vocabulary based on expression levels, while the second is from the gene ontology vocabulary of cellular categories. This study employed 11 different vocabularies, yielding a total of about 5,000 descriptors (see Table S1 in the supplemental material). Given this information, redescription mining aims to find subsets of the high-expressor gene set that exhibit concerted behavior in at least two different vocabularies. Unions, intersections, and differences (set-theoretic constructs) are allowed in either/both of the vocabularies, in an attempt to find equivalence relationships (niches) involving the descriptors. Neither the subsets nor the ways that the descriptors are combined are inputs to the automated algorithm. The quality of the redescription is based upon Jaccard's coefficient, the ratio of genes that conform to the redescription to those that participate in either of the descriptors but not necessarily in both. All coefficients are listed over the redescription arrows in Fig. 4 and 5, with the algorithm using a cutoff threshold of 0.5. A typical analysis yields numerous redescriptions, and each of these must be evaluated individually.

Using redescription for understanding desiccation tolerance. Redescription R1 imposed a trend on the descriptor set showing genes with increasing expression after rehydration (Fig. 4) and redescribed them in relation to genes up-regulated during heat shock but down-regulated during peroxide treatment. Three genes were identified, i.e., *SIP18*, *GRE1*, and a putative γ-glutamyl kinase gene, *YHR033W*. *SIP18* and *GRE1* were two of the most up-regulated genes found in our study (Fig. 2). Sip18p and Gre1p share those properties used to define a larger group of proteins, the hydrophilins (22), but possess no strong mutual similarities except for their N termini. A FASTA-based search of the N-terminal 18-mers in the nonredundant database identified similar N termini in putative proteins from *Kluyveromyces lactis* and *Candida glabrata*, as well as a dehydrin-like protein from the desiccation-tolerant resurrection plant *Selaginella lepidophylla*. *SIP18* may be part of an osmotic stress response locus on chromosome XIII consisting of *ALD2*, *PAI3*, and *SIP18*, with regulation occurring through the *HOG2* signaling cascade (43) as well as through the cyclin-dependent kinase Ssn3p (Srb10p) (28). Analysis of Sip18p demonstrated that the carboxy-terminal lysine residues of the protein are essential for binding to phospholipids in vitro (54). Transcription of *GRE1* and two other genes, *GRE2* and *GRE3*, was reported to be responsive to osmotic and other stresses (21). In our experiments *GRE2* was activated twofold upon desiccation and then its expression decreased during

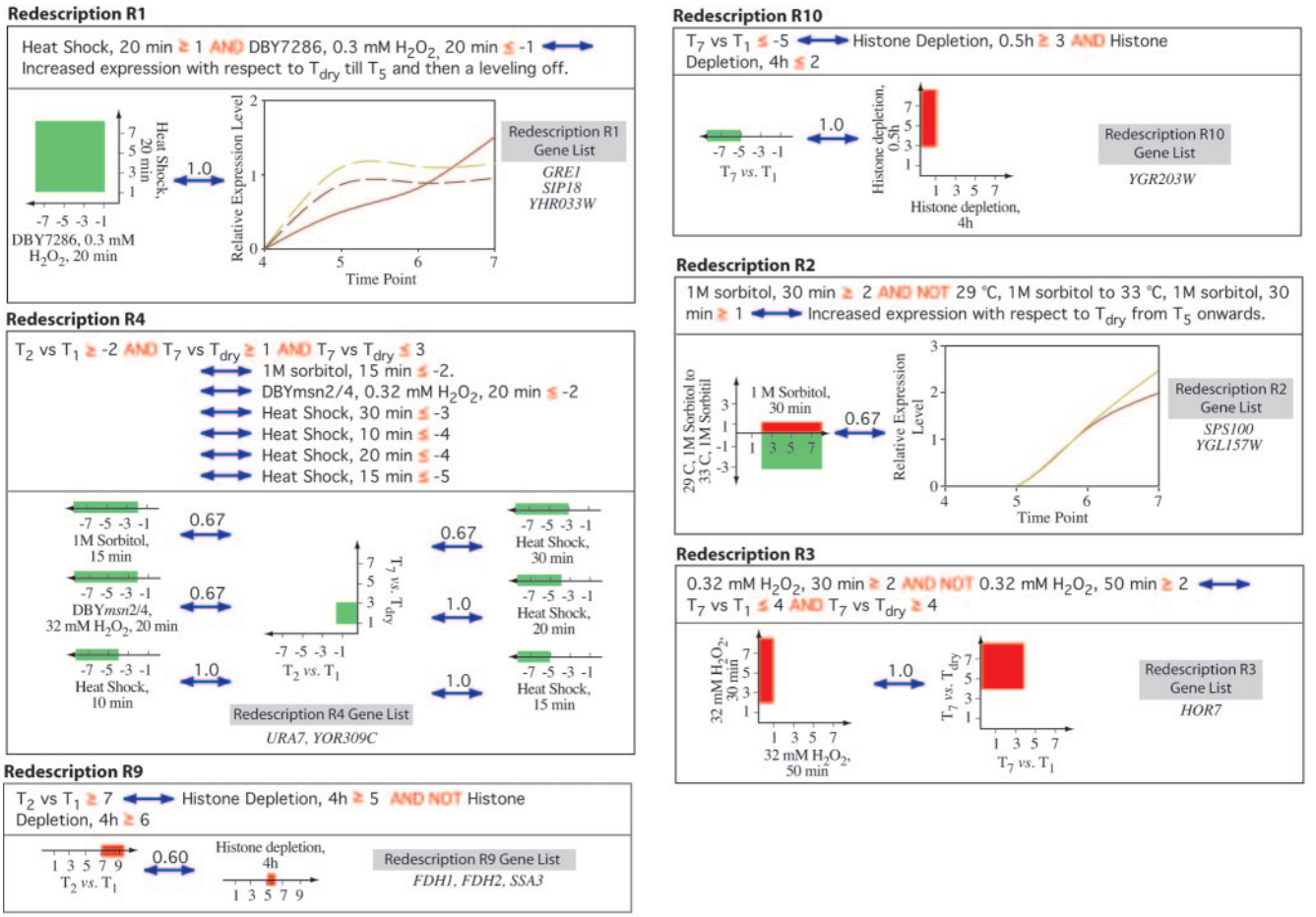

FIG. 4. Redescriptional analysis of desiccation and rehydration in yeast. The top panel of each redescription describes the redescription in a text format. The bottom panel shows the redescription in graphical format. The Jaccard coefficient for each redescription (redescription quality) is shown over the redescription (blue) arrow. Also included in the bottom panels is the gene list obtained from the redescription. Limiters are in place for fold changes so that they do not pass through a fold change of zero. Thus, when a fold change states greater than −2, a range from −2 to 0 is meant. If a region defined in the graphical format extends to the end of the axis, it indicates that genes above the highest value listed on the axis will still be included (i.e., there is no upper limit of the fold change value). Notice that some redescriptions, such as R4, are multiway and provide more than two ways to describe a given set of genes. Redescriptions R9 and R10 compare the BY4743 data with those reported from the histone depletion study of Wyrick et al. (65); all others utilize data from Gasch et al. (23).

rehydration; transcription of *GRE3* showed no activation in response to either stress. Gre2p was reported to interact with Pwp1p and Ygr111wp (30). Pwp1p is a β-transducin-like protein containing WD-40 repeats characteristic of F-box-like proteins in many organisms (31). The latter proteins are implicated in control of cell cycle transition, transcriptional regulation, and signal transduction (see below). Ygr111wp interacts with Lys14p (30), a transcriptional activator of the lysine pathway genes (with 2-aminoadiptate semialdehyde as a coinducer), and is involved in saccharopine reductase synthesis.

Redescription R4 identified two additional candidate desiccation-regulated genes. *URA7* (CTP synthetase) and *YOR309C* (unknown function) had transcription profiles of marked down-regulation at T_2 and up-regulation following rehydration. CTP synthetase plays an essential role in the synthesis of all membrane phospholipids in eukaryotic cells, including yeast (42, 48). *YOR309C* encodes a 16-kDa protein with lysine and arginine contents of 16.7 and 27.8%, respectively, which is similar to the case for the nucleic acid binding protamine encoded by *YOR053W*. Control of chromatin condensation is likely an important component of the drying response of cells (59), and this is a potential role for *YOR309C*. In an effort to look at heterochromatin/euchromatin relationships, we attempted to redescribe the desiccation and rehydration data with those of a histone depletion study (65). In redescription R9, two formate dehydrogenase genes (*FDH1* and *FDH2*) along with a heat shock protein-encoding gene (*SSA3*) were significantly up-regulated in both studies, and in another case (redescription R10), a putative tyrosine phosphatase gene (*YGR203W*) was found to be down-regulated during desiccation and rehydration but increased in expression during the initial phase of the histone depletion experiment.

Recovery/rehydration phase. In order to obtain additional information on genes that may be important in the rehydration phase, the rehydration time points (T_4 to T_7) were compared with T_{dry} (desiccated cells), as opposed to T_1. The expression levels of only a very small subset of genes were up-regulated as

high as fourfold in this comparison. The elevated expression levels of *TUB3* and *SRO9* suggest that potential changes in the cytoskeleton may occur in the rehydration phase. *TUB3* encodes α-tubulin and is involved in homologous chromosome and mitotic segregation and nuclear migration. Sro9p is involved in the organization of actin filaments and associates with translating ribosomes (polysomes). With respect to the latter, three genes, *S22B*, *S16B*, and *S26B*, encoding ribosomal proteins of the 48S initiation complex, were up-regulated 3.1- to 3.7-fold during rehydration.

Redescription was also used to investigate the rehydration process. Redescription R2 (Fig. 4) associates genes that are increased in transcription level during recovery (relative to T_{dry}) with those associated with a 1 M sorbitol stress. Two genes were identified: *SPS100* and *YGL157W*. *YGL157W* was down-regulated overall relative to T_1, but its level increased significantly during the rehydration phase. Interestingly, this gene is annotated as encoding a protein with similarity to plant dihydroflavonol 4-reductase, the corresponding function of the protein encoded by *GRE2*. *SPS100*, up-regulated more than sevenfold, encodes a sporulation-specific wall maturation protein, Sps100p, which has a protective role during the early stages of spore wall formation (34), presumably when wall reorganization is occurring.

In redescription R3, *HOR7*, a hyperosmolarity-responsive gene, was up-regulated almost 10-fold at 6 h of rehydration (relative to T_{dry}); the same gene was induced 7-fold in the presence of 0.32 mM H_2O_2 for 30 min but not for 50 min (Fig. 4). Hor7p is a small, depolarizing plasma membrane-bound protein (36). It shares 49% sequence identity with Ddr2p, a protein currently annotated as a DNA damage response heat shock protein. Ddr2p has been suggested to be a vaculolar membrane protein (18), and it is highly probable that is serves a role similar to that of Hor7p in the vacuole membrane. The mode of expression of *HOR7* suggests a role in the rehydration response, thereby suggesting that depolarization of the plasma membrane may be a physiological response to rehydration.

Desiccation tolerance and sulfur metabolism. Redescription R5 (Fig. 5) states that "among the 210 high expressors found in our study with BY4743, the only genes that are onefold or more down-regulated during heat shock (30 min) (23) are also those genes that are between one- and fivefold down-regulated at T_2." This redescription relates the expression changes of yeast during desiccation to those occurring during heat shock (23). A conspicuous feature of redescription R7 is the presence of three genes involved in sulfur metabolism: *SAM1* (S-adenosylmethionine synthetase gene), encoding the protein that synthesizes the potential riboswitch ligand S-adenosylmethionine (12), *SAH1* (*S*-adenosyl-L-homocysteine hydrolase gene), and *YFR055W* (cystathionine β-lyase gene). Riboswitch ligands such as SAM appear to serve as ancient master control molecules whose concentrations are monitored to ensure homeostasis of a much wider set of metabolic pathways (61, 64), and indeed SAM has recently been implicated in G_1 cell cycle regulation (45).

The results of a redescription can be used to further evaluate responses by determining the known interactions of the proteins encoded by these three genes. Sam1p was reported to interact with 13 other proteins (24), and the gene for one of these, *URA7*, is also present in the redescription. In an iterative process using each of the genes (and their respective protein interactions) (9), a network of interactions was assembled from the redescription set (Fig. 5C). Assembly of the network also relied upon the primary microarray data to infer possible additional relationships. For example, *MET30*, encoding a cell cycle F-box protein and also involved in sulfur metabolism and protein ubiquitination, can directly or indirectly be associated with *TEF4* (translation elongation factor EF-1γ gene) and *CLN2* (cyclin-dependent protein kinase regulator gene), both of which are present in the redescription. Note that *MET30* itself was not present in the redescription, and in fact it had the highest level of up-regulation of all cell cycle control genes. Further refinement of the network included the clustering of genes from a pathway that shows interactions with other genes in the redescription but not with one another (for example, the clustering of *HIS4* and *HIS1*, *ARO1* and *ARO4*, *LYS14* and *LYS 12*, and *GAS1* and *GAS3*). With the exception of *MET30*, *SIP18*, and *CDC34*, the transcription of each gene in the proposed network was either down-regulated or unchanged, sug-

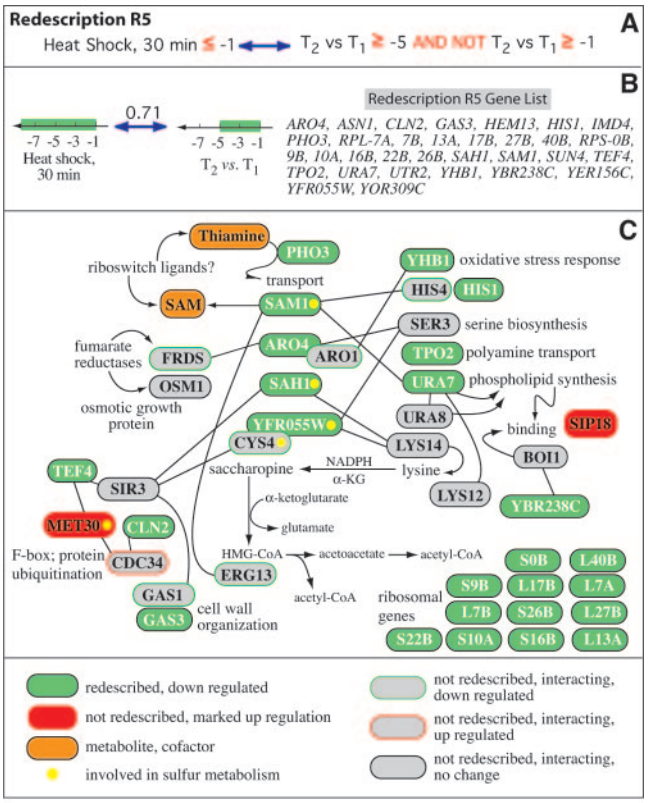

FIG. 5. Redescriptional analysis and sulfur metabolism. A, Statement of redescription R5 that relates heat shock to the desiccation experiment. B, Graphical depiction of the redescription and the genes identified. C, Desiccation and heat shock lead to down-regulation of sets of genes with a central function in sulfur metabolism. Genes present in the redescription were first analyzed to determine if their products had any known interaction with one another (e.g., *SAM1* and *URA7*) or with other proteins (e.g., *CLN2* and *CDC34*), The network of interactions and functions was built in an iterative fashion; in view of the potential function of its protein in phospholipid binding, *SIP18* is shown close to other genes associated with lipid synthesis and binding (*URA7* and *YBL085W*).

TABLE 1. Extra- and intracellular glucose and trehalose levels during desiccation and rehydration

Location	Compound	Concn[a] during:						
		Desiccation			Rehydration			
		T_1 (control)	T_2 (18 h)	T_{dry}	T_4 (15 min)	T_5 (45 min)	T_6 (90 min)	T_7 (360 min)
Medium[b]	Glucose	1.15 ± 0.43	0.22 ± 0.20	2.80 ± 0.18	3.10 ± 0.66	2.7 ± 0.49	2.42 ± 0.26	0.35 ± 0.16
	Trehalose	0.07 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.09 ± 0.01
Cells	Glucose	<0.1	<0.1	2.5 ± 1.0	<0.1	<0.1	<0.1	<0.1
	Trehalose	<0.1	0.35 ± 0.12	0.40 ± 0.25	0.35 ± 0.11	0.31 ± 0.14	0.34 ± 0.16	0.69 ± 0.24

[a] Data are averages and standard deviations from triplicate biological experiments with duplicate analyses per time point (six values per time point). Values are expressed in milligrams per plate for medium and milligrams per gram of dry cells for cells.
[b] Medium was separated from cells, freeze-dried, and taken up in water (5 ml). The initial volumes at T_1 and T_4 were 15 ml.

gesting a role for sulfur metabolism in the desiccation and rehydration response.

Medium and cellular levels of glucose and trehalose. The medium and cellular concentrations of glucose and trehalose are shown in Table 1. As the medium volume was variable over the course of the experiment, the extracellular concentrations of glucose and trehalose are given on a plate basis (the initial volume at T_1 was 15 ml). Glucose concentrations dropped rapidly during the evaporative phase but increased when the cells were completely dry (T_{dry}), presumably due to the release of free glucose from dead/lysed cells. The concentration then remained the same during the majority of the rehydration phase, decreasing dramatically between 90 and 360 min. As mentioned above, cell cycle analysis indicated that a normal FACS profile was not observed until 44 h after rehydration when cells were rehydrated in 1/20 YPD. Trehalose concentrations in the medium remained constant over the duration of the experiment.

The cellular concentration of glucose was above the limit of detection only in the dry sample (T_{dry}). The prevailing viewpoint in the yeast literature is that the intracellular concentration of glucose is very low. We attribute the measurable level of intracellular glucose to be "apparent intracellular glucose," i.e., glucose that is actually extracellular and bound to the cell wall. The cellular trehalose level was increased at the T_2 time point and held relatively steady through desiccation and rehydration, exhibiting a measurable increase at T_7.

DISCUSSION

Wine yeast is rarely found on grapes with an unbroken skin, presumably due to lack of moisture and nutrients, but viable cells are found inside approximately one-third of damaged berries, where colonies establish fermentation chambers (46). Desiccated colonies entrapped within fragments of dried berries are potentially an important source of inocula following dispersal (11, 47). In this context, it is important to emphasize that there are numerous permutations of environmental conditions under which *S. cerevisiae* could be subjected to water stress. Thus, a number of empirical decisions were made in designing our experiments to dry, desiccate, and rehydrate yeast, including decisions as to the stage of growth of the cells, the time and temperature of drying, the length of time of desiccation, and the mode of rehydration. The fact that there was substantial overlap in the transcriptional analysis of *S. cerevisiae* BY4743 and a sample of commercially dried yeast, purchased at random (Fig. 2), suggests that the stress responses reported here are of environmental relevance.

The fatty acid catabolism transcriptome was activated markedly under the conditions employed, most prominently the genes of peroxisomal biogenesis and protein targeting, fatty acid transport and activation, beta-oxidation, and peroxisome-mitochondrion acetyl-CoA shuttling. It is highly probable that this shift was due solely to the glucose-limiting conditions needed to dry the cells. However, at some point in time during the desiccation process, the cells will no longer be able to take up glucose, even when supplies are adequate, and must begin to shut down all processes. Our working hypothesis is that this shutdown procedure is coordinated and controlled. What is not known is the sequence of events leading to metabolic arrest. If glucose uptake is repressed early in this process, it may be that the fatty acid catabolism transcriptome will be up-regulated even when adequate supplies of glucose are present.

The interplay between pyruvate and formate and the role of hypoxic and glucose-limiting conditions during desiccation and rehydration need further investigation. Based upon results presented here, as well as the recently reported data for the mitochondria of potato tubers (10), we hypothesize that a formate-producing pathway is present in yeast as well as other eukaryotes and is an important contributor to cell energetics under quiescent conditions. In bacteria, pyruvate is converted to formate and acetyl-CoA by PFL, a free radical-generating enzyme (3). However, as the induction of formate dehydrogenase has been reported to occur under normoxic, glucose-limiting conditions (7) and the PFLs found in the past are oxygen sensitive, an enzyme with an alternative mechanism may be at work.

We propose that under the glucose-limiting conditions employed in this study, trehalose is not available in the amounts necessary to provide significant biophysical protection of the membranes. An increase in trehalose levels was observed, and appeared to be increasing at the latter stages of rehydration, but the levels found were significantly lower than that generally reported for membrane protection of yeast (53). Similar responses at the transcriptional level for trehalose and glycerol-related genes have been reported under glucose-limiting conditions (20), suggesting that the response may be related to the amount of carbohydrate available to the organism. Limiting supplies, as either free sugar or stored glycogen, may make the

overall cost of trehalose biosynthesis through gluconeogenesis too expensive. Trehalose may be produced during desiccation only when yeast is exposed to osmotic stress with ample glucose supplies.

Glycerol is the major osmolyte synthesized under hyperosmotic stress and is responsible for most of the osmotic adjustment in yeast (1). Glycerol-3-phosphate dehydrogenase, encoded by *GPD1*, is responsible for glycerol synthesis, and lack of the enzyme renders the yeast osmosensitive. Transcription of *GPD1* was unaffected through T_6 and then down-regulated at T_7, providing evidence for distinct differences in the responses of yeast to osmotic and matric water stress. There are examples of anhydrophiles that do not rely upon trehalose for survival. The cyanobacterium *Nostoc commune*, for example, produces an extracellular polysaccharide in combination with a water stress protein to survive desiccation (51), rather than producing significant quantities of trehalose. Bdelloid rotifers have also been shown to undergo anhydrobiosis without producing trehalose (33).

Because there is no significant biosynthesis of trehalose or glycerol in desiccated yeast, we propose that a peptide or small protein such as the hydrophilin Sip18p could also function in a similar role. *SIP18* was the most up-regulated gene in our experiments, and its product is known to bind to phospholipid (54). The broad class of proteins referred to as hydrophilins include *l*ate *e*mbryogenesis *a*bundant (LEA) proteins and dehydrins and are characterized by their high hydrophilicities (>1.0) and high glycine (>6%) contents (22). These criteria appear to correlate strongly with the transcriptional response of a particular hydrophilin gene to hyperosmotic conditions, and LEA proteins are implicated in providing desiccation tolerance to plants and plant seeds by an as-yet-unknown mechanism. A recent search of the yeast genome revealed 12 potential hydrophilin-like genes, and analysis of their transcript levels upon osmotic stress (not desiccation) indicated that 8 of them were up-regulated (22). The Yeast Genome Database (18) currently lists 10 genes under the category "response to desiccation." However, in our studies, transcription of only 3 of the 10 listed genes was markedly enhanced during desiccation and rehydration: *YJL144W* (up to 3-fold), *YPL223C* (*GRE1*) (up to 7.6-fold) and *YMR175W* (*SIP18*) (up to 9.3-fold). Genes encoding phospholipases A_2, B, C, and D showed no to only a slight change in level of transcription during the course of the experiment, in contrast to the marked elevation in the transcription of genes involved with fatty acid beta-oxidation. These observations may suggest that there is minimal to no mobilization of fatty acids from membranes of viable cells to fuel beta-oxidation.

One of the findings from our analyses was the change in transcription of genes involved in cell wall structure and organization (down-regulation of *GAS1*, *GAS3*, *FKS1*, and protein *O*-mannosyltransferase genes *PMT1* to *-5*) and lipid synthesis and binding (down-regulation of *SER3*, *URA7*, *URA8*, *YBL085W*, and *YBR238C*), in addition to down-regulation of *TGL1*, encoding the major lipid particle protein. Fourteen of the 19 proteins identified by a proteomic analysis of tryptically digested cell walls of log-phase *S. cerevisiae* (66) were down-regulated in our study at least twofold. In contrast, there was up-regulation of cell wall spore-specific genes *YNL196C* and *SPS100*. These data suggest that cell wall organization and composition are critical for desiccation tolerance. A reduction in cell wall rigidity may allow the cell to distribute stabilizing factors in a more effective manner.

In summary, we have presented the first transcriptional analysis of *S. cerevisiae* during desiccation and rehydration. The sets of tools, resources, and data that have been generated will allow other laboratories to extend our understanding beyond the initial discoveries and hypotheses presented here. Knowledge of the structural, physiological, and molecular bases for desiccation tolerance will contribute to our basic understanding of the living cell and its inherent ability to enter into, and return from, a state of complete metabolic arrest. In addition, we believe our studies lay a strong foundation for the development of new biomimetic strategies for long-term storage of labile cells and cell components. A desiccation-like strategy may be extremely useful for the long-term goal of placing sensitive cells, of relevance to the biomedical and biodefense communities (4, 6, 16, 26), in a state of full metabolic arrest. Applications include circumventing the requirement for refrigerated storage used with vaccines and blood products, development of robust biosensors, and the long-term preservation and archiving of valuable cell lines and clone libraries.

ACKNOWLEDGMENTS

This work was funded through the Multidisciplinary University Research Initiative (MURI) program of the Department of Defense (Biomimetic Cell and Tissue Stasis; N00014-01-1-0852) as well as the Metabolic Engineering for Cellular Stasis program of DARPA (N00173-98-1-G005-P00004 and N00173-02-1-G016).

We are grateful to Bud Mishra, Keithanne Mockaitis, and Walter Niehaus for critically reading the manuscript.

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